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SOYBEAN CYST NEMATODE - IDENTIFICATION AND EXTRACTION TECHNIQUES

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A major factor limiting soybean production in Iowa is parasitism by the soybean cyst nematode, *Heterodera glycines*. Soybean cyst nematode was identified in Grundy, Mahaska, and Ringgold Counties for the first time in 1996 and is now known to be present in 72 Iowa counties. It is very likely that the nematode is present in many other counties as well, but the nonspecific nature of the above-ground symptoms of soybean cyst nematode damage makes early identification or diagnosis of infestations difficult.

Above-ground symptoms of soybean cyst nematode damage often do not appear consistently, and may be absent for several years following initial infestation. The primary above-ground symptoms of soybean cyst nematode damage are chlorosis or yellowing and stunting of the soybean plants. These symptoms are not unique and often can be attributed to damage due to iron deficiency chlorosis, other nutrient deficiencies, drought stress, herbicide injury, compaction, or other plant diseases. In many instances, injury due to soybean cyst nematode probably has gone undetected because of lack of above-ground symptoms or misdiagnosis of these nondescript symptoms. Symptoms can range from slight to severe depending on the age and vigor of the soybean plants, the population density of the nematode in the soil, the level of soil fertility and moisture, and other environmental conditions. Consequently, one should not rely upon above-ground symptoms for conclusive identification of a soybean cyst nematode infestation.

Field Identification

The only unique sign of an infestation of soybean cyst nematode is the appearance of adult females and cysts of the nematode on the soybean roots. Females and cysts appear as tiny lemon-shaped objects which vary in color from cream to yellow to tan to brown, depending on the age of the nematode. They are large enough to be seen with the unaided eye, but observation with a magnifying glass usually is easier. The cysts and adult females are about the size of the period at the end of this sentence, considerably smaller than the nitrogen-fixing nodules on the roots. Roots should be dug, not pulled, from the soil in the field, otherwise many of the females and cysts may become dislodged. Observation of the adult females and cysts on the roots of infected soybean plants is the ONLY conclusive way to diagnose infestations of soybean cyst nematode in the field.

If soybean yields in a particular field have leveled off or steadily decreased for no apparent reason or if soybean cyst nematode females and cysts are observed on soybean roots in the field, soil sampling and analysis are warranted.

Soil Sampling Strategies

Generally, soil samples are collected and analyzed for soybean cyst nematode for one of three reasons: to determine whether the nematode is present in a field, to determine if the nematode is causing observed stunted and/or yellowed plants, and to determine the extent (population density) of an infestation in a

field known to have the nematode. These three approaches to sampling are referred to as scouting, diagnostic, and predictive sampling, respectively.

Scouting Sampling

Because above-ground symptoms of soybean cyst nematode may not become apparent for years, growers farming in areas where the nematode is prevalent should consider collecting soil samples as part of a routine scouting program. Quite often, previously undetected soybean cyst nematode infestations are discovered as a result of such scouting efforts. These newly discovered infestation generally have lower population densities than infestations causing obvious above-ground symptoms, and, consequently, management of nematode infestations discovered by scouting usually is much easier. Fields that warrant the most immediate attention for sampling are fields cropped to continuous soybean or numerous years of soybean in the recent past, fields that have produced gradually diminishing soybean yields over the past several years for no apparent reason, fields that have been recently flooded and may have been contaminated with soil from an adjacent field or farm, and fields that have been worked in by custom applicators or with borrowed equipment. When soil sampling for scouting purposes, soil should be collected from areas of the field that are likely to be first infested with the nematode. These "high-risk" areas include areas of the field where equipment enter, along fence lines where wind-blown soil accumulates, low spots of the field where surface water accumulates, or in areas of the field where unthrifty soybean growth had been observed in the past.

Diagnostic Sampling

Diagnostic soil sampling is performed when the soybean crop is in the field and the plants are showing obvious above-ground symptoms. When sampling for diagnostic purposes, one should collect two separate samples, one from the problem area and another from a nearby area which does not appear to be affected. Also, soil should be collected from near plants showing the most dramatic symptoms as well as from near some that are not as severely affected.

Predictive Sampling

Predictive soil sampling is performed in an attempt to gain information on the severity of a known soybean cyst nematode infestation for use in making management decisions for the upcoming growing season. Predictive soil sampling usually is done in the fall after the crops have been harvested, or in early spring prior to planting. Individuals should sample fields that are going to be used for soybeans in the upcoming growing season. Information gained from predictive soil samples taken in the spring probably is slightly more accurate than that obtained from fall samples, but there is much less time to contemplate management strategies based on the results of spring samples. If sampling for predictive purposes, collect the soil in a systematic, zigzag pattern within the area. Limit the area sampled to no more than 15 - 20 acres; if a larger field is to be sampled, divide the field into 15 - 20 acre parcels and collect separate samples from each parcel. Define the parcels of land to be sampled based on agronomic parameters such as soil type, pH, drainage, elevation, or prior cropping history. The fewer the number of acres represented in each sample, the more accurate and representative the results will be.

General Soil Sampling Guidelines

Some general guidelines that should be followed when taking scouting, diagnostic, or predictive soil samples for soybean cyst nematode are:

1. Use a 1-inch-diameter soil probe, if available, or a small hand shovel to collect the soil. At 15 to 20 different places within the sampling area, collect a soil core or 1/4 cup of soil with a shovel. Soil should be collected to a depth of 6 to 8 inches, ideally from near the base of the plants if they are still present. All of the soil representing a single area should be placed in a bucket and mixed very thoroughly. From the well-mixed soil, remove approximately one pint to send in for processing.

2. The soils to be tested should be placed in plastic or wax-coated paper bags to prevent drying and should be kept cool. Avoid storing the samples in the sun, and ship the samples as soon as possible. Several facilities throughout the state and region will analyze soil samples for soybean cyst nematode. Soil samples can be sent to Iowa State University for soybean cyst nematode analysis. Samples should be sent to the Plant Disease Clinic, 323 Bessey Hall, Department of Plant Pathology, Iowa State University, Ames, IA 50011. Please include the following information on Form PD-32 for each sample sent to Iowa State University:

    a. Name, address, and telephone number of grower and collector.
    b. County and town nearest to where the sample was collected.
    c. Estimated acreage that the sample represents.
    d. Cropping history of the area sampled.
    e. Current crop in the area sampled.
    f. Test desired is a soybean cyst nematode egg count

Current fees for the analysis of soil samples will be indicated on the back of Form PD-32. Copies of this form can be obtained from county extension offices or the Publications Distribution Office at Iowa State University (telephone number 515-294-5247).

Extraction Techniques

Following is an outline of the techniques used by the Iowa State University Plant Disease Clinic for determination of soybean cyst nematode population densities from soil samples. The procedure has three stages: extraction of the cysts from the soil, crushing of the cysts to release the eggs, and microscopic observation of the suspension of eggs for counting.

Extraction of cysts from soil:

The technique used to recover the cysts of soybean cyst nematode from soil is a combination of wet-sieving and decanting. It is a modification of a mycological technique used to recover large spores of soil-inhabiting fungi (Gerdemann, 1955) and is based on the fact that the size range for soybean cyst nematode cysts is 470 - 790 μm by 210 - 580 μm. The procedure is as follows:

1. Obtain a well mixed 100 cc soil sample (approx. 1/2 cup).

2. Fill a bucket with 2 quarts of water.

3. Pour the soil into the water, break any clumps with your fingers, and mix the soil suspension well for 15 seconds.

4. Let the suspension settle for 15 seconds.

5. Pour the soil suspension through an 8-inch-diameter #20 (850 μm pore) sieve nested over a #60 (250 μm pore) sieve. Any sediments that settle out in the bottom of the bucket should be discarded.

6. Rinse, with water, the debris caught on the top sieve, then discard its contents. Carefully wash the cysts and accompanying sediments trapped on the #60 sieve into a clean, properly labeled beaker OR directly into a 100 ml polypropylene grinding tube, using as little water as possible.

Extraction of eggs from the cysts:

The result of the above technique is a suspension of SCN cysts, along with organic debris and sediments similar in size to the cysts, that could be counted using a simple dissecting microscope. In fact, some laboratories that analyze soil for soybean cyst nematode report results in the form of cyst per 100 cc of soil. It is the opinion of many researchers, including those at Iowa State University, that the number of eggs contained within each cyst varies too much for cyst counts to be used as a reliable measure of the soybean cyst nematode population density. Therefore, Iowa State University does not perform cyst counts for soybean cyst nematode. Instead, eggs are extracted from cysts, and results are reported in the form of eggs per 100 cc of soil.

Eggs of soybean cyst nematode average 47 μm by 100 μm in size. The procedure used at Iowa State University to crush cysts to release and recover the eggs is as follows (Niblack et al, 1993):

1. Wash the cyst suspension from the beaker into a 100 ml polypropylene grinding tube. Do not fill the tube more than half full.

2. Grind the cysts carefully between the inside surface of the tube and the 1-mm-deep grooves on a stainless steel pestle attached to a Talboys Model 101 motorized laboratory stirrer. Grind the cysts for exactly 60 seconds at 3,500 RPM. Rinse the pestle thoroughly with a wash bottle when finished grinding.

   Alternatively, cysts may be crushed in a blender for 60 seconds at medium speed, provided a small canister is used atop the blender. The blender canister should hold no more than 500 ml or so for blending to be effective in rupturing the cysts.

3. After grinding or rupturing the cysts, pour the suspension in the tube or blender canister through a stainless steel, 3-inch-diameter #200 (75 μm pore) sieve over a #500 (25 μm pore) sieve. Rinse the tube or canister several times with tap water, each time pouring the contents through the sieves. Carefully rinse with water the sediments caught on the #200 sieve, then discard. Finally, carefully wash sediments and eggs caught on the #500 sieve into a clean beaker with as little water as possible.

4. Place 4 to 5 drops of egg stain into each beaker of egg suspension. Egg stain consists of 3.5 g acid fuchsin dissolved in a solution of 250 ml acetic acid and 750 ml water (Byrd et al., 1983).

5. Heat beakers containing the eggs and stain in the microwave oven on full power until the suspension begins to boil. Several beakers of eggs can be microwaved at one time.

Counting the eggs:

After the egg suspensions cool, eggs can be observed with a dissecting microscope and either a specially made nematode counting slide or a rectangular counting dish.

A. Counting eggs with the nematode counting slide

The volume of the egg suspension in the beaker should be brought up to exactly 50 or 100 ml with tap water. Fill the chamber of the nematode counting slide with a well-mixed suspension of the stained eggs using a Pasteur pipette. The specially made nematode counting slides are constructed so that the volume of egg suspension observed over the grid is exactly one ml. Consequently, simply count the number of stained eggs that appear within the grid of the slide to determine the number of eggs per ml of suspension. The total number of eggs in the sample can then be calculated by multiplying the number of eggs per ml by the total volume of the stained egg suspension (50 or 100 ml).

B. Counting eggs with rectangular counting dishes

Inexpensive counting dishes can be made by scratching four narrow lanes in the bottom or top half of clear plastic rectangular hinged boxes using a sharp dissecting needle. The total area of the four lanes should equal half of the total area of the bottom of the hinged box.

To determine the number of eggs in a sample, add tap water to the beaker of stained eggs until the total volume is exactly 50 or 100 ml. Pipette a known volume of well-mixed egg suspension (2 to 5 ml) into the rectangular counting dish, count the number of eggs in the four lanes, and multiply by two to get the total number of eggs per volume originally added to the dish. The total number of eggs in the sample then can be calculated by dividing the total number of eggs counted by the volume added to the dish (2 to 5 ml) then multiplying the number of eggs per ml by the total volume of the stained egg suspension (50 or 100 ml).

The surface of the egg suspension in the counting dish may be sprayed with 95% ethanol to break surface tension and allow for more rapid settling of the eggs.

Literature Cited

Byrd, D. W., Jr., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissue for detection of nematodes. Journal of Nematology 15:142-143.

Gerdemann, J. W. 1955. Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. Mycologia 47:619-632.

Niblack, T.L., R.D. Heinz, G.S. Smith, and P.A. Donald. 1993. Distribution, density, and diversity of *Heterodera glycines* in Missouri. Supplement to the Journal of Nematology 25: 880-886.

Sources of Materials and Equipment

I. Soil sampling probes:

Various types of 1-inch-diameter soil sampling probes can be ordered from the following companies:

Ben Meadows Company, 3589 Broad Street, P.O. Box 80549, Atlanta, GA 30366

Clements Associates, Inc., R.R.#1 Box 186, Newton, IA 50208
(515) 792-8285

Forestry Suppliers, Inc., 205 W. Rankin Street, P.O. Box 8397, Jackson, MS 39284-8397
(601) 354-3565

Oakfield Apparatus Inc., P.O. Box 65, Oakfield, WI 53065
(414) 583-4114

II. Sieves:

Sieves may be purchased through either of the following distributors:

Fisher Scientific, 1600 W. Glenlake Avenue, Itasca, IL 60143
(800) 223-9114

VWR Scientific, P.O. Box 66929, O'Hare AMF, Chicago, IL 60666
(800) 932-5000

Note: The brass, 8-inch-diameter #20 and #60 sieves generally are in stock, but the stainless steel, 3-inch-diameter #200 and #500 sieves probably will have to be special-ordered through the distributor.

III. Polypropylene 100 ml grinding tubes:

Nalgene round lip, polypropylene, 100 ml centrifuge tubes can be ordered through Fisher Scientific (cat. # 05-562-10C, pkg. of 10).

IV. Stainless steel pestle with 1mm ridges:

The stainless steel pestle used to grind the cysts is custom manufactured for our purposes by the Iowa State University Engineering Research Institute (ERI) Machine Shop. The ERI Machine Shop will make this item for individuals outside of the university as well. Contact the ERI Machine Shop, 124 ERI Building, Iowa State University, Ames, IA 50011 for further details. Remember to mention that the pestle is to be identical to those previously made at the facility for Dr. Gregory Tylka in the Department of Plant Pathology.

V. Motorized stirrer for stainless steel pestle:

The motorized laboratory stirrer used with the stainless steel pestle is a Talboys Model 101 stirrer. This stirrer can be purchased through VWR Scientific or directly through Talboys Engineering Corporation, South Montrose, PA 18843.

VI. Nematode counting slides:

The specially made nematode counting slides can be purchased from Olympic Equine Products, 5004 228th Avenue S.E., Issaquah, Washington 98027, (296) 391-1169.

VII. Rectangular counting dishes:

Rectangular counting dishes can be made from clear plastic boxes obtained from Althor Products, 496 Danbury Road, Wilton, CT 06897. Order catalog # H-12, 2 7/8" x 1 3/16" x 1" clear polystyrene hinged boxes, singly or in packages of 100 (makes 200 dishes).

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